Multivalent carboxylic acids to modify the properties of zein ☆

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Abstract

Methods must be developed to improve both the processability and physical properties of zein-based articles. Typical plasticizing agents that improve processability have a negative impact on physical properties at elevated humidities. Continued efforts must be employed to discover improved plasticizers. Carboxylic acids having more than one carboxylic acid moiety have been evaluated in zein melt formulations produced in a torque rheometer. These reagents were effective plasticizers for zein, lowering zein viscosity, and delaying the onset of rapid viscosity increase. These reagents altered viscosity differently than the traditional plasticizers such as triethylene glycol. These additives also lowered the tensile strength (TS) of zein formulations at 50% RH. Surprisingly, TS at 70% RH was higher than that at 50% RH at elevated levels of these additives. NMR analysis of Soxhlet extracted samples showed that these multivalent carboxylic acids do not cross-link the zein under the reaction conditions employed.
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1. Introduction

Developing higher valued products from the coproducts of the bioethanol industry would improve the economics of this market. Zein is the main protein in corn and is one of the main coproducts of this industry. Zein makes up approximately 6% of the corn kernel and approximately 33% of the potential coproducts. Efforts have been put forth to reduce the cost for isolating zein (Shukla et al., 2000). Zein products typically have physical properties that are inferior to those prod-

ucts based on petroleum. Additional research is required to produce zein-based articles that have physical properties that are less affected by humidity. Historically, cross-linking, typically with formaldehyde, has been one method to improve the physical properties at both low and high humidities (Yelland, 1951; Jenkins and Magee, 1958; Uy, 1998). Industrial use of formaldehyde requires significant investment to handle it safely. Other cross-linking technologies have been described, but these utilize materials that would also require significant investment to perform safely industrially (Wu et al., 2003; Kim et al., 2004). Other methods must be developed to improve the physical properties of zein and reduce the impact of moisture so that zein can be used more widely. Polycarboxylic and multivalent acids have been used to improve the wet strength of cellulosic materials, presumably through cross-linking reactions (Yang and Xu, 1998; Xu and Yang, 1999; Kim et al., 2000). Citric acid has been used to modify the metal binding properties of

soybean and corn protein in the melt state, but their physical properties were not assessed (Sessa and Wing, 1998). Multivalent acids, such as citric acid (CA) and 1,2,3,4-butanetetracarboxylic acid (BTCA), have been used as cross-linking reagents for zein in solution and provided improved physical properties (Yang et al., 1996). These reagents presumably react with the terminal amine or the hydroxyl moieties on serine or threonine. However, these additives have not been evaluated in a melt process to improve the physical properties. Furthermore, it was not determined how these formulations performed when the products are exposed to high humidity.

Melt processing provides many commercial advantages relative to solution chemistry, but melt extrusion of zein has had mixed results (Uy, 1996; Zhang et al., 1997). In order to process zein successfully, plasticizers are required (Selling et al., 2004). With the addition of plasticizers, tensile strength was found to decrease as the amount of plasticizer increased. Reductions in tensile strength became larger at higher humidities (Lawton, 2004). Additives such as tri(ethylene glycol) and glycerol that make the zein more hydrophilic gave larger reductions in tensile strength at higher relative humidities (Lawton, 2004).

An inherently safer technology to improve physical properties as produced as well as physical property retention at elevated humidity is required. The technology should be amenable to economic melt processing. Improved plasticizers must be found that will improve melt processing of zein and have a smaller impact on physical properties, especially tensile strength, at higher humidities. We have studied the use of multivalent carboxylic acids in a zein melt process to determine how these reagents affect melt rheology and improve the tensile strength at different humidities.

2. Materials and methods

2.1. Materials and equipment

Zein grade F4000 was obtained from Freeman Industries LLC, Tuckahoe, NY, and used as received (Lot F40003121C, 91.50% protein, 5.0% fat, 0.04% fiber, and 0.05% ash). Moisture content of the supplied zein was measured at various times during testing and ranged between 3.5 and 6.0%. Deionized water was used to adjust moistures to the desired level. Tri(ethylene glycol) (TEG), ethanol, methyl sulfoxide-d_6 (DMSO-d_6), sodium hypophosphite, CA, and BTCA were used as received from Aldrich Chemical Company, Milwaukee, WI. A Haake Fisons, Rheocord 90 torque rheometer, with the 600 mixing bowl (Thermo Electron Corpora-

tion, Madison, WI) was used to make the torque measurements on the blends. Torque curves presented have been smoothed using 1.5 min moving averages. High shear roller rotors were used and set to either 50 or 65 rpm. Initial temperature of the chamber walls of the torque rheometer was 90 or 105 °C. The temperature of the blend while being sheared was not controlled. A Carver Model C press (Wabash, IN) was used to make compression molded tensile bars 6.3 cm long, 9 mm wide at the widest point, and 3 mm wide at the narrowest point. Sample thicknesses were measured at five different locations using a micrometer (Model No. 49–63, Testing Machines Inc., Amityville, NY). Constant relative humidity (RH) at 25 °C was obtained using Hotpack Humidity Chambers (Warminster, PA). Moisture contents were measured on an Ohaus moisture meter MB45 (Pine Brook, NJ). Tensile strength (TS), Young's modulus (YM), and elongation to break (Elo) were evaluated for each sample using an Instron Universal Testing Machine Model 4201. Physical properties were determined with a crosshead speed of 50 mm/min, a gauge length of 7.62 mm, and a 1 kg load cell. NMR spectra were run in DMSO-d_6 on a Bruker 500 MHz NMR (Billerica, MA).

2.2. Blend preparation and mixing

Zein blends were prepared similar to that described by Selling et al. (2004). The reagents were added to 50 g zein (dry basis) and the blend was initially stirred with a spatula to provide a crude mixture. The order of addition was not a factor. Water was evaluated as a plasticizer at 15, 23, or 26% (dry basis). TEG was evaluated as a plasticizer at 10% (dry basis). This mixture could be added to the Haake rheometer in approximately 2 min. The start time ($T = 0$) for all tests was 1 min after complete addition of the reagents.

For the production of compression molded samples, the dough produced from the Haake was frozen with liquid nitrogen and then ground to a powder. The mold could produce four test samples at a time. The powder (1.5 g) was placed into each mold. Molding conditions were selected to provide a tensile test sample that did not have visible remnants of the starting powder. The mold was heated at 138 °C and pressure of 17 MPa, except where noted, for 20 min and then allowed to cool at room temperature. Steam that formed during molding could escape through the narrow gaps in the mold.

2.3. Soxhlet extraction

Approximately 10 g of finely ground melt processed zein incorporating 15% water, 10% TEG, and 10% CA

as plasticizers was loaded into a cellulose thimble. This sample was then placed in a Soxhlet extractor using ethanol (200 ml) as the solvent. The extraction process was run for 7 h. The zein sample was removed from the thimble, dried, and analyzed (NMR) in DMSO-d_6, with a control sample taken from the zein before treatment.

2.4. Sample characterization

Samples used for physical property analyses were stored for a minimum of one week at either 50 or 70% RH. The moisture contents for samples after storage at these conditions were measured by heating the samples in an Ohaus moisture meter MB45 at 105 °C for 20 min. For all sample sets, tensile runs were performed on either three or four samples. Values reported are mean values.

3. Results and discussion

3.1. Function of multivalent acids—additive or cross-linker

Before extensive studies were undertaken utilizing these reagents, we focused on whether the multivalent carboxylic acids formed chemical bonds with the zein to yield branch points or cross-linking, or whether they were incorporated as additives. Soxhlet extraction experiments were employed to determine how the multivalent carboxylic acids were incorporated into the zein formulations. A zein formulation composed of 15% water, 10%

TEG, 10% CA, and 5% sodium hypophosphite was produced on the torque rheometer. Sodium hypophosphite has been used as cross-linking promoter with zein when using multivalent acids of this type in solution (Yang et al., 1996). The resulting dough was finely ground and then extracted using ethanol on a Soxhlet extractor. The NMR spectra of the zein formulation in DMSO-d_6 are presented in Fig. 1. The doublet of doublets at 2.6 ppm are the methylene hydrogens of citric acid or its ester (Fig. 1A). It is difficult to determine whether the citric acid is bound to the zein from this spectrum. After Soxhlet extraction, the NMR spectrum of the zein formulation does not display these peaks (Fig. 1B). The same results were obtained when the sodium hypophosphite was not included in the formulation. The citric acid is present in the zein as an additive and is not bound to the zein. By analogy, BTCA is also present as an additive only. The presence of hydrophilic additives has been shown to be detrimental to physical properties (Lawton, 2004), but when the additives were salts, the effects were very large (Sessa et al., 2005). Thus, sodium hypophosphite was not used in conjunction with the multivalent acids so that these reagents were evaluated as plasticizers only.

3.2. Melt rheology

To determine whether these types of reagents were effective plasticizers, various amounts of the multivalent carboxylic acids were mixed with zein and then processed in a torque rheometer. This procedure has been
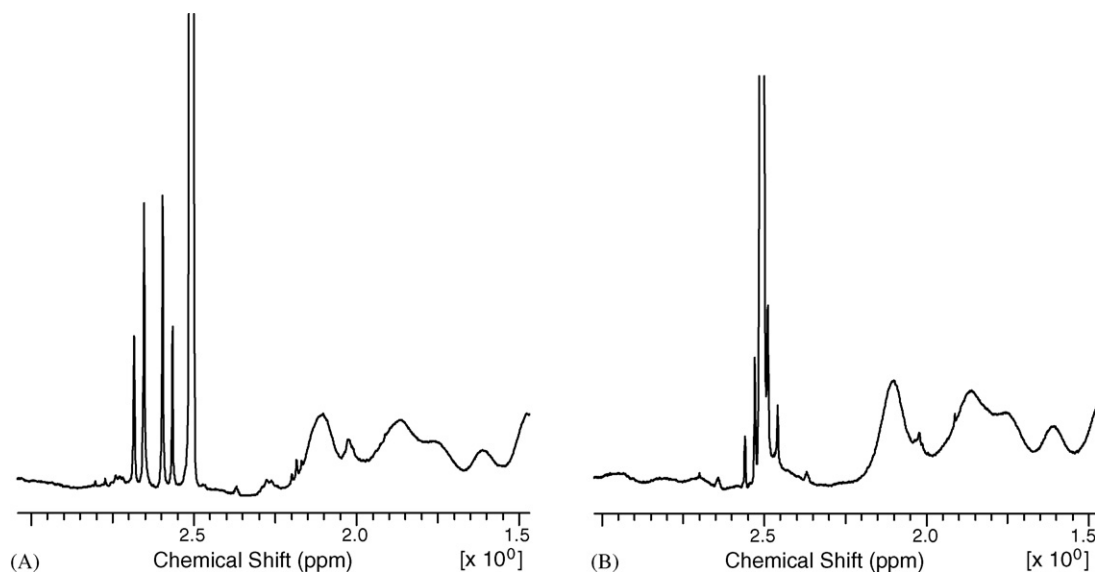

Fig. 1. NMR proton spectra of zein formulation including sodium hypophosphite in DMSO-d_6, before (A) and after (B) Soxhlet extraction. Signals at 2.6 ppm are from citric acid residues. Note disappearance of signals at 2.6 ppm after extraction.
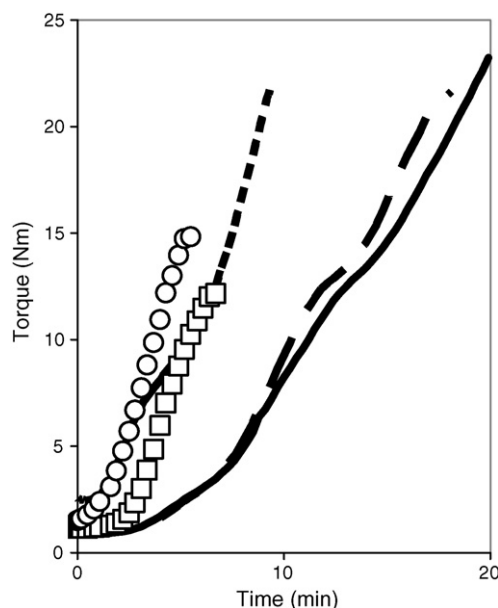
Fig. 2. Effect of either 5% BTCA or CA on rate of torque increase of zein. Rheometer run at 50 rpm using roller rotors. Line designation and composition: (- - -) 26% total water at 90 °C, (–––) 26% total water + 5% BTCA at 90 °C, (—) 26% total water + 5% CA at 90 °C, (○) 26% total water at 105 °C, and (□) 26% total water + 5% BTCA at 105 °C.

shown to be a good method for defining the rheology of a melt (Selling et al., 2004). When the amount of BTCA or CA was 10% or less, another reagent, such as water or TEG, was needed in order to process the zein. As these multivalent carboxylic acids are relatively high melting, they would not provide sufficient lubricity to the zein during initial processing. On addition of either 5% BTCA or CA to a zein mixture having 26% water, the initial torque of the mixture was reduced (Fig. 2). The amount of water was not optimized. The amount of water used was such to give an initial torque value of approximately five or less. Torque traces were stopped when the dough began to wrap the roller rotors resulting in inaccurate torque readings. The large difference that is readily observed in these traces is the time when the rapid torque increase occurred. This delay in the time at which the torque increase had been previously observed in zein blends where sodium dodecylsulfate had been used in conjunction with water and/or TEG (Sessa et al., 2005). Polyvinyl chloride (PVC) mixtures processed on the same equipment also produced torque curves with very similar shapes (Chen et al., 1995; Tomaszewska et al., 2004). In these studies, the rapid rise in torque is attributed to PVC gelation and the resulting rapid viscosity rise. Gelation is defined as that process where the small amorphous domains of PVC begin to flow, and the

polymer chains then entangle and form microcrystalline domains. The formation of microcrystalline domains of PVC is analogous to protein aggregation. The presence of plasticizers will slow the rate at which the entanglements will take place and retard the resultant viscosity rise. The formation of protein–protein bonds cannot be ruled out as a contributor to increased viscosity. Loss of water with time at elevated temperature will generate a gradual increase in torque. However, it has been shown previously that during the time when the rapid viscosity increase takes place, the amount of water present in the sample does not change significantly (Selling et al., 2004).

When the temperature of the melt is increased, the same rapid torque increase was observed (Fig. 2). When the control formulation containing zein with 26% total water is run at an initial temperature of 105 °C, the rapid torque increase takes place at an earlier time and at a faster rate. This temperature effect has been observed previously (Selling et al., 2004). With the addition of 5% BTCA to this formulation at 105 °C, the time at which the rapid torque increase takes place is once again delayed. The torque increase for the formulation including BTCA at 105 °C occurs earlier than this same formulation when run at 90 °C. The rate of toque rise for the BTCA formulation at 105 °C is also higher than the same formulation at 90 °C.

When the amount of multivalent carboxylic acid is increased, the time at which the rapid torque rise takes place is delayed. This is detailed for BTCA in Fig. 3. This effect is to be expected if these reagents are acting as plasticizers. The presence of higher quantities of plasticizer will slow the rate of aggregation.

TEG is a plasticizer used with zein that has been shown to improve elongation (Lawton, 2004). When equal molar amounts of BTCA or CA are added to zein blends incorporating both water and TEG as plasticizers, the rapid viscosity rise is once again delayed relative to formulation where the multivalent acid is not present (Fig. 4). To determine whether the effect is simply due to the presence of additional plasticizer, an experiment was run where additional TEG was added to provide an equivalent total molar amount of plasticizer relative to 10% TEG with 5% BTCA or 4% CA. To achieve this amount of plasticizer, 13.2% TEG was used in addition to 15% total water and processed on the torque rheometer. This formulation undergoes its rapid viscosity rise at an even earlier time than when 10% TEG is used (Fig. 4). This phenomenon where additional plasticizer gives rise to an earlier rapid viscosity rise has been observed previously (Selling et al., 2004). While the higher amount if plasticizer reduces initial viscosity, this same lower
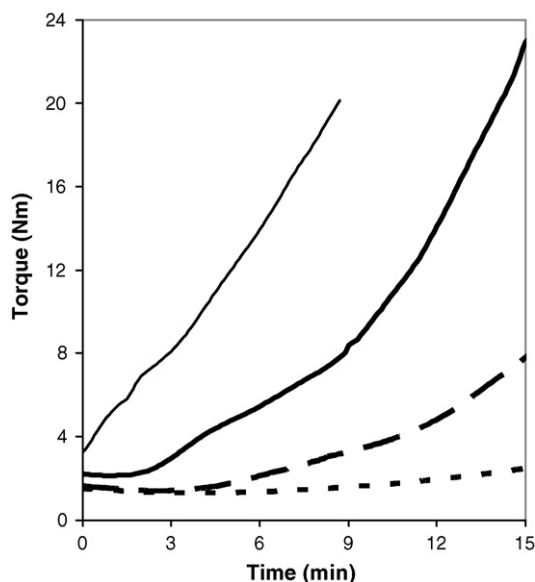
Fig. 3. Effect of increasing amounts of BTCA on rate of torque increase of zein. Rheometer run at 65 rpm, 90 °C block temperature using roller rotors. Line designation and composition: (–) 23% total water, (—) 23% total water + 5% BTCA, (– – –) 23% total water + 10% BTCA, and (- - -) 23% total water + 15% BTCA.
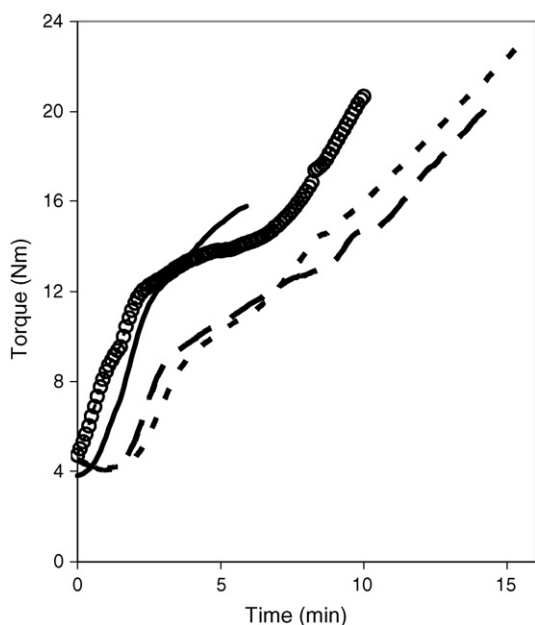

Fig. 4. Effect of combinations of TEG and water with/without 5% BTCA or 4% CA on torque increase for zein formulations. Rheometer run at 50 rpm, 90 °C block temperature using roller rotors. Line designation and composition: (—) 10% TEG + 15% total water, (○) 13.2% TEG + 15% total water, (– – –) 10% TEG + 15% total water + 5% BTCA, and (- - -) 10% TEG + 15% total water + 4% CA.

viscosity allows the protein molecules to align more readily giving aggregation and rapid viscosity growth. This demonstrates that the multivalent acids impact zein rheology in a different fashion than plasticizers such as TEG. It has been previously observed in corn gluten meal that additives such as water, glycerol, octanoic acid, and dibutyl tartrate interact with different parts of the protein (Di Gioia and Guilbert, 1999). Mixtures of these plasticizers were not evaluated. Information is needed to understand how variations in plasticizer chemistry affect zein rheology.

3.3. Physical properties

The impact that these reagents have on the physical properties of melt processed zein formulations has not been determined. The properties of various formulations incorporating BTCA or CA are detailed in Table 1. As with most plasticizers, as the amount of BTCA or CA increases, TS decreases. With the addition of as small as 2.5% BTCA, TS decreases from 24.7 to 19.3 MPa. The load–elongation plot has the typical behavior of brittle materials (Fig. 5). At elevated amounts of BTCA, TS decreases significantly from 24.7 to 3.8 MPa at 70% RH. In general, as the relative humidity increases, TS decreases significantly for zein formulations containing hydrophilic additives (Lawton, 2004) It is interesting to note that at elevated levels of BTCA, the TS of the zein formulations at 70% RH is actually higher than at 50%. This phenomenon, where TS is higher at elevated humidities, has not been observed previously for zein formulations. For formulations using CA, the same trend is obtained. As the amount of CA is increased, TS decreases by 70% or more, but at the highest level of CA
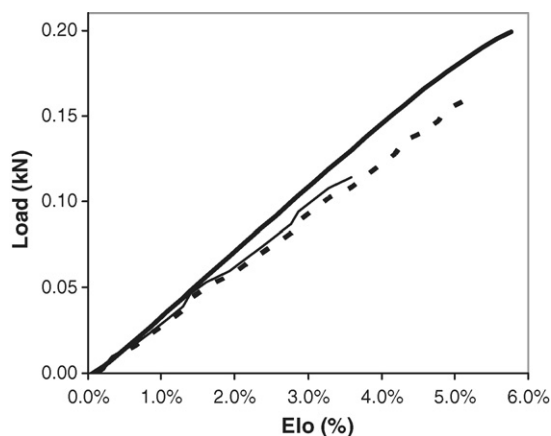

Fig. 5. Load–elongation plot for: (—) 10% TEG + 15% total water, (- - -) 10% TEG + 15% total water + 5% BTCA, and (–) 10% TEG + 15% total water + 4% CA.

Table 1
Effect of BTCA and CA on zein physical properties

Sample	RH[a] (%)	Moisture (%)	TS (MPa)	TS S.D.	Elo (%)	Elo S.D.	YM (MPa)	YM S.D.
10% TEG + 15% total water	50	4.2	24.7	1.3	6.9	0.3	422	29
(control)	70	6.4	19.0	1.0	6.9	0.4	328	19
10% TEG + 15% total water + 2.5% BTCA	50	3.4	19.3	0.6	5.3	0.1	400	21
	70	6.6	18.5	2.1	7.5	0.8	280	17
10% TEG + 15% total water + 3.4% BTCA	50	4.0	18.1	3.2	4.3	0.8	470	26
	70	6.8	19.2	1.3	7.9	0.3	290	19
10% TEG + 15% total water + 5% BTCA	50	4.0	17.5	1.4	5.2	0.5	369	18
	70	6.8	17.6	1.4	11.2	1.2	202	20
10% TEG + 15% total water + 6% BTCA	50	3.0	16.4	2.1	3.8	0.7	498	30
	70	5.2	15.9	2.6	7.0	1.3	258	16
10% TEG + 15% total water + 10% BTCA	50	3.8	5.5	0.8	2.2	0.2	281	21
	70	6.2	9.3	2.5	7.4	2.0	150	19
10% TEG + 15% total water + 2.8% CA	50	4.4	19.5	0.9	5.1	0.6	430	27
	70	6.6	17.1	1.6	7.1	1.1	288	14
10% TEG + 15% total water + 4.2% CA	50	4.2	14.6	1.6	3.7	0.3	445	44
	70	5.6	17.6	3.2	7.2	1.8	288	12
10% TEG + 15% total water + 10% CA	50	4.0	7.3	1.1	2.7	0.2	326	14
	70	8.2	9.8	1.2	9.8	1.6	138	5

[a] RH, relative humidity.

tested, the TS samples stored at 70% RH is higher than the TS of samples stored at 50% RH. Improvements in wet strength has been previously observed in cellulosic materials using multivalent acids (Yang and Xu, 1998; Xu and Yang, 1999; Kim et al., 2000).

Based on the unusual impact that higher levels of BTCA or CA have on physical properties at elevated humidities, efforts were expended to understand the mechanism for the improved properties. IR spectra and solid-state NMR spectroscopy were performed to determine whether there was a hydrogen bonding network forming. For the IR spectra, the carbonyl stretches were examined carefully and no differences were observed between the test and control. Similarly the solids-state proton NMR spectra did not show differences between test and control. Additional efforts are being put forth to better understand how these multivalent acids are interacting with zein at high relative humidities leading to improved TS.

4. Conclusions

Citric acid and 1,2,3,4-butanetetracarboxylic acid are effective plasticizers, lowering initial viscosity and delaying the onset of the fast torque increase. CA and BTCA behave in a different manner than TEG, in that they lengthen the time it takes for the rapid viscosity rise to take place. These materials do not act as cross-linking reagent under the conditions employed. As the amount of either additive is increased, the TS decreases at 50% RH. At the highest incorporation levels, the TS at 70% RH is higher than that at lower humidity. This phenomenon is unusual and warrants future investigation.

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